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# Analysis of National Animal Health Monitoring System Tonsil Samples for Presence of ail-bearing Yersinia enterocolitica by using a 5′ Nuclease Fluorogenic PCR (TaqMan) Assay

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**Abstract**
The fluorogenic 5′ nuclease polymerase chain reaction (TaqMan) assay, which was developed in this laboratory, was further optimized and used to screen hogs for Yersinia enterocolitica (4). Tonsil samples (n = 1,647) collected during the National Animal Health Monitoring System (NAHMS) Swine 2001 program were tested for the presence of the ail gene, which is present in virulent Y. enterocolitca. Tonsil swabs were collected on farms (n = 101) located in Arkansas, Colorado, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Dakota, Texas and Wisconsin. Of the 1,647 tonsil samples, 11.4% (n = 188) were positive.

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# Analysis of National Animal Health Monitoring System Tonsil Samples for Presence of *ail*-bearing *Yersinia enterocolitica* by using a 5´ Nuclease Fluorogenic PCR (TaqMan) Assay

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**Summary and Implications**

The fluorogenic 5′ nuclease polymerase chain reaction (TaqMan) assay, which was developed in this laboratory, was further optimized and used to screen hogs for *Yersinia enterocolitica* (4). Tonsil samples (n = 1,647) collected during the National Animal Health Monitoring System (NAHMS) Swine 2001 program were tested for the presence of the *ail* gene, which is present in virulent *Y. enterocolitca*. Tonsil swabs were collected on farms (n = 101) located in Arkansas, Colorado, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Dakota, Texas and Wisconsin. Of the 1,647 tonsil samples, 11.4% (n = 188) were positive.

## Introduction

*Y. enterocolitica* is a major human foodborne pathogen which accounts for ~87,000 cases each year in the United States. Yersiniosis is reported frequently in cooler climates (1,5). The most prevalent clinical signs, especially in children, are abdominal pain, fever, diarrhea, nausea, and vomiting (3). Post-infection symptoms include erythema nodosum and reactive arthritis. Swine are an important animal reservoir of *Y. enterocolitica* strains, including serotypes 0:3 and 0:9, which are pathogenic in humans (1,2,10,11). In one study, virulent strains of *Y. enterocolitica* 0:3 were found in the oral cavity and the gastrointestinal tract of pigs (10). In the United States, serotypes 0:8 and 0:5,27 predominate (1,2,3,5,10,11).

Two *Y. enterocolitica* genes, *inv* and *ail*, play a major role in the ability of this microorganism to enter mammalian cells (9,11).

The fluorogenic polymerase chain reaction (TaqMan) assay is a PCR-based protocol that by-passes post-PCR processing for the detection of the PCR product. The 5´ PCR assay uses the 5´ ➪ 3´ activity of *Thermus aquaticus* DNA polymerase (11) and requires a fluorogenic probe. TaqMan fluorogenic probes consists of an oligonucleotide with reporter dye at the 5´ end and a quencher dye at the 3´ end. When the probe is intact, the quencher dye suppresses the reporter (6). If the target DNA sequence is present, the probe anneals downstream from one of the primer sites and it is cleaved by the DNA Poly merase as the primer is extended. This cleavage liberates the reporter dye from the quencher dye. The liberated reporter dye increases fluorescenses (6,7,11). The increase in fluorogenic signal is a direct consequence of the successful PCR amplification of specific DNA targets (11).

## Materials and Methods

*Sample Enrichment Procedure***.** Copan tonsil swabs were collected from hogs (n = 1,647). Swabs were enriched in 10 ml ITC media (2 days at room temperature) after which 100 µl of ITC was streaked to CIN (2 days at room temperature).

*DNA Extraction Procedure.* DNA extractions were prepared from all the ITC samples as follows. A 1 ml aliquot from the ITC media was centrifuged (2 minutes, 10,000 x g). The supernatant was carefully removed and the remaining pellet was resuspended in 200 µl of PrepMan sample preparation reagent (PE Applied Biosystems, Foster City, CA). The tubes were vortexed to resuspend the pellet and then heated (102 °C for 10 minutes). The tubes were centrifuged (2 minutes at 10,000 x g) to pellet cell debris. The supernatant was transferred into a new microcentrifuge tube and was labeled with the kit and sample number. DNA was also extracted from the presumptive *Yersinia* colonies on the CIN plates. DNA extractions were stored at -20 °C until PCR analysis.

*Primer and Probes.* The probe and primer sequences target the *Y. enterocolitica ail* gene (4; Table 1). Oligonucleotide primers and the fluorescently labeled probe were synthesized by Integrated DNA Technologies (Coralville, IA). The probe was labeled at the 5′ end with fluorescent reporter dye FAM-6-carboxy-fluorescein and the 3′ end with the quencher dye TAMRA-6-carboxy-tetramethyl-rhodamine.

*Fluorogenic 5´Nuclease PCR Conditions.* The PCR conditions were described previously (4). Each PCR mixture contained 3.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 1.25 U of AmpliTaq Gold DNA polymerase (PE Applied Biosystems), and 1 X GeneAmp PCR Gold Buffer (150mM Tris-HCl, 500 mM KCl) (PE Applied Biosystems). 5 microliters of DNA template was added to yield a total volume of 50µl. Concentrations of primers (200 mM) and the probe (25 nM) as originally described (4) yielded inconsistent results. Therefore, primers (50 to 900 nM) were tested in

a matrix format with a known positive DNA template. Optimal concentrations of 300 nM for both the forward and reverse primer were then used to screen the tonsil samples. Probe concentration was also increased from 25 to 200 nM. The thermal cycling conditions were as follows: 95° C for 10 minutes and 35 cycles of 95° C for 15 seconds (denaturation) and 58° C for 1 minute (primer annealing and extension) followed by an indefinite hold at 25° C (4).

Analysis was completed on the ABI 7700 Sequence Detection System. Cycle 33 was set as an arbitrary cut off point. Any sample that crossed the threshold prior to cycle 33 and showed logarithmic amplification was scored as positive. Any sample that crossed the threshold after or on cycle 33 or did not show sufficient amplification was scored as negative (Fig 1).

**Results and Discussion**

The *ail* gene of pathogenic *Y. enterocolitica* was detected in 11.1% of ITC samples (124 of 1,120) and in 12.1% (64 of 527) of samples plated to CIN (Fig 1).

The fluorogenic PCR assay detected the *ail*-bearing strain of *Y. enterocolitica* in tonsil swabs of normal market-weight hogs. This is evidenced by the 11.1% positive ITC samples and 12.1% positive CIN samples. Swine are known carriers of *Y. enterocolitica.* This study confirmed that virulent species of *Y. enterocolitica* harboring the *ail* gene are present in tonsil swabs of United States swine.

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**Table 1. List of primers and probe used in the assay.**

| Primer/probe | Annealing site | Sequence (5′ ➪ 3′) |
|---|---|---|
| TM1-F | 746 to 772 | GGT CAT GGT GAT GTT GAT TAC TAT TAC |
| | | |
| TM1-R | 818 to 836 | CGG CCC CCA GTA ATC CAT A |
| | | |
| TM1-P | 785 to 814 | CCA TCT TTC CGC ATC AAC GAA TAT CTT AGC |